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RESEARCH ARTICLE

In vitro antiglycation and hypoglycaemic effects of *Syzygium cumini* leaf extracts

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Abstract: Glycation is a prime mechanism responsible for chronic diabetic complications, a process which is enhanced under hyperglycaemia. Natural inhibitors of protein glycation and those which can lower postprandial blood glucose elevation are of utmost importance in minimising the damage caused by diabetes. One objective of this study was to assess the *in vitro* inhibitory effects of *Syzygium cumini* (SC) leaf extracts on protein glycation and α-glucosidase activity. The other objective was to identify the type of inhibition on α-amylase activity. SC leaf powder was sequentially extracted with hexane (H), ethyl acetate (E), methanol (M) and water (W). *In vitro* inhibitory effects of extracts on protein glycation, α-amylase and α-glucosidase enzymes were measured. M and W were the major fractions recovered [50.74 and 27.94 % (w/w), respectively] while H was the smallest fraction [7.35 % (w/w)] out of total yield. All extracts inhibited fructosamine formation, protein glycation and protein cross-linking at 2 $mgmL^{-1}$. At 0.05 $mgmL^{-1}$, fructosamine formation was inhibited in the presence of E, M and W while there was no significant inhibition with H. At 0.1 $mgmL^{-1}$, antiglycation effect of the H extract was negligible while other extracts retained their effects. IC_{50} values of H, E, M and W extracts against α-glucosidase were 7.89, 3.11, 0.86 and 0.69 μgmL^{-1}, respectively. A mixed type inhibitory effect was observed on α-amylase with E, M and W extracts. H extract did not inhibit α-amylase. In conclusion, the results provide evidence for antiglycation and hypoglycaemic effects of SC leaf extracts demonstrating better activities in E, M and W extracts.

Keywords: α-amylase, α-glucosidase, glycation, inhibitors, *Syzygium cumini*.

INTRODUCTION

A primary target of diabetes management aims at lowering the blood glucose concentration as hyperglycaemia is a salient feature of diabetes (Sheard *et al*., 2004). Reduction of postprandial blood glucose elevation is one mechanism available in bringing down the blood glucose concentration. Activity of the pancreatic α-amylase and α-glucosidases of the intestinal brush border are essential in hydrolysing dietary starch into absorbable products (Kumar *et al*., 2011; Sales *et al*., 2012). Hence, α-amylase and α-glucosidase inhibitors serve as important avenues in the management of blood glucose concentration in diabetic patients (Alagesan *et al*., 2012a).

One of the detrimental effects of elevation of blood glucose concentration is the acceleration of non-enzymatic glycation of biomolecules as the rate of glycation depends on the concentration of sugar in the medium. Protein glycation is implicated as a main cause for the development of chronic diabetic complications (Goh & Cooper, 2008). Long-lived proteins such as collagen are more prone to glycation induced damage (Aronson, 2003). During early glycation reactions, sugar molecules react with free amino groups of proteins forming stable Amadori products such as fructosamine (Meeprom *et al*., 2013). Further reactions of glycation, which occur over a longer period result in the production

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of a heterogeneous group of irreversible compounds known as advance glycation end products (AGEs) (Abdallah *et al*., 2016). During later stages, AGEs can lead into intra or inter molecular cross-linking. Hence, AGEs are responsible for causing permanent structural and functional damage to the affected molecules (Sadowska-Bartosz & Bartosz, 2015).

Plants with antidiabetic properties have been used since ancient times all over the world as an effective, inexpensive and safe mode of treating diabetes (Modak *et al*., 2007). Although more than 1000 plant species with hypoglycaemic activity have been recognised (Grover *et al*., 2002), most of them are used without a proper scientific validation (Jung *et al*., 2006).

Syzygium cumini (L.) Skeels (Myrtaceae) Syn.: *Eugenia jambolana* Lam., *Myrtus cumini* L., *Syzygium jambolanum* DC. and *Eugenia cumini* (L.) Druce (local name: *Madan*) is a large tree, widely distributed in South Asian countries such as Sri Lanka (Ayyanar & Subash-Babu, 2012). Almost all the parts of *S. cumini* (SC) are used to treat various illnesses including diabetes. The seeds and bark of SC are used as one of the most effective treatments of diabetes (Jung *et al.*, 2006). Most investigations carried out on validating the antidiabetic effects of SC were targeting on the seed and bark (Poongunran *et al*., 2016). Only limited data are available on the hypoglycaemic and antiglycation effects of SC leaves although it is a source more widely available throughout the year compared to that of seed and bark from this evergreen species.

One objective of this study was to assess the *in vitro* inhibitory effects of *Syzygium cumini* (SC) leaf extracts on protein glycation and α-glucosidase activity and the other objective was to identify the type of inhibition on α-amylase activity.

METHODOLOGY

Plant material

Syzygium cumini leaves were collected from Jaffna District, Sri Lanka. The plant was authenticated by the Deputy Director, National Herbarium, Royal Botanical Gardens and the voucher specimen (HKIP-JKP-BIO-2013-01) was deposited at the Royal Botanical Gardens, Peradeniya, Sri Lanka.

Duration of the study was from January 2014 to October 2016.

Preparation of SC leaf extracts

SC leaves were collected, cleaned and dried under shade. The dried leaves were powdered using a grinder and the leaf powder was sequentially extracted with hexane (H), ethyl acetate (E), methanol (M) and water (W). Another batch of leaf powder was extracted directly in methanol as a crude extract (C) for glycation experiments. Solvents were filtered and evaporated under low temperature using a rotary evaporator to yield dry extracts. The dry extracts were dissolved in phosphate buffer with or without dimethyl sulfoxide (DMSO) to the required concentrations prior to the assays.

Estimation of relative fructosamine concentration

The relative concentration of fructosamine was quantified using protein and sugar incubation mixtures as described by Perera *et al*. (2016). Briefly, chicken egg lysozyme (Sigma) was incubated with fructose (500 mM) in phosphate buffer (pH 7.4, 200 mM, 0.02 % sodium azide). Incubations were carried out in the presence or absence of 2, 0.25 and 0.05 $mgmL^{-1}$ SC leaf extracts at 37 ºC for 5 days.

Aminoguanidine (1 $mgmL^{-1}$) (AG) was used as the standard inhibitor. Test blanks and control blanks were prepared in the absence of fructose. Aliquots collected on day 5 were analysed for the reduction of nitroblue tetrazolium. Test samples were mixed with the sodium carbonate buffer (pH 10.35, 0.1 M) and left for 5 min at 37 ºC. Fructose (500 mM) was added to the test blanks and control blanks just before the assay. Nitroblue tetrazolium (0.5 M) in sodium carbonate buffer (pH 10.35, 0.1 M) was added to each tube and incubated for 15 min at 37 ºC. The absorbance at 530 nm was measured immediately after incubation. Percentage inhibition of the relative fructosamine concentration in the presence of SC extracts and AG was calculated. Estimations were performed 3 times on separate occasions in duplicate.

Percentage inhibition of relative fructosamine formation was calculated using the following formula.

$$\text{Percentage inhibition} = 100 - \left\{\frac{(\text{Absorbance of test} - \text{Absorbance of test blank}) \times 100}{(\text{Absorbance of control} - \text{Absorbance of control blank})}\right\}$$

Assessment of the degree of glycation

Glycation inhibitory effects of SC extracts were assessed using bovine serum albumin (BSA) *in vitro* as described

by Wijetunge and Perera (2014). In summary, BSA was mixed with fructose (500 mM) in phosphate buffer (pH 7.4, 200 mM, 0.02 % sodium azide). SC leaf extracts were added to the reaction mixtures at 5, 1 and 0.1 $mgmL^{-1}$. AG (1 $mgmL^{-1}$) was used as the positive control. Corresponding blanks were prepared in the absence of fructose. The reaction mixtures were incubated at 37 ºC for 30 ds and aliquots were collected at day 12 or 13 and day 30 for further analysis. Degree of glycation in each aliquot was assessed by conducting polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions using Enduro vertical gel electrophoresis system-E2010-P. Standard Laemmli method was followed using 10 % polyacrylamide gels (Laemmli, 1970). Gels stained with Coomassie brilliant blue were monitored for the changes in the migration position of the BSA bands. The approximate percentage inhibition of glycation was assessed based on the decrease in migration of BSA in the presence of SC extracts (in comparison to the uninhibited reaction). Experiments were repeated 3 times.

Assessment of the degree of glycation induced protein cross-linking

Glycation induced protein cross-linking inhibitory effect of SC extracts was assessed according to the method described by Perera and Ranasinghe (2015). In summary, chicken egg lysozyme was mixed with fructose (500 mM) in phosphate buffer (pH 7.4, 200 mM, 0.02 % sodium azide). SC leaf extracts were added to the reaction mixtures at 2, 0.5, 0.25 or 0.1 $mgmL^{-1}$. AG (1 $mgmL^{-1}$) was used as the positive control. Corresponding blanks were prepared in the absence of fructose. The reaction mixtures were incubated at 37 ºC for 21 ds and aliquots were collected on day 5, 7 and 21 for further analysis using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). Standard Laemmli method was conducted with Enduro vertical gel electrophoresis system- E2010-P, using 12 % SDS-polyacrylamide gels. Gels stained with Coomassie brilliant blue were monitored for the inhibitory effects on the appearance of high molecular weight products of lysozyme. Experiments were repeated 3 times.

Effects of SC extracts on α-glucosidase activity

Measurement of α-glucosidase activity

Inhibitory effects of the SC leaf extracts against α-glucosidase were determined using the method by Elya *et al*. (2012) with slight modifications (Perera *et al*., 2016). In brief, 200 µL of 67 mM sodium phosphate buffer (pH 6.8) and 120 µL of 10 mM *p*-nitrophenyl α-D-glucopyranoside were mixed. SC extract (40 µL) was added to the test and test blank to reach a final concentration of 10 μgmL^{-1}. Mixtures were pre-incubated for 15 min at 37 ºC. α-Glucosidase (0.1 U) was added to the tests and control (40 µL) and the reaction mixtures were incubated for 15 min at 37 ºC. After 15 min, 200 mM sodium carbonate (800 µL) was added to terminate the reaction. The amount of *p*-nitrophenol released was measured at 405 nm. Percentage inhibition of α-glucosidase was calculated with the formula used to measure the percentage inhibition of relative fructosamine formation. Estimations were performed 3 times on separate occasions in duplicate.

The concentration of the extract that inhibits 50 % of the glucosidase activity (IC_{50}) was calculated using a series of concentrations. IC_{50} values were determined using a graph plotted with percentage inhibition (Y axis) and $\log_{10}$ extract concentration (X axis) (Sudha *et al*., 2011).

Kinetics of α-amylase inhibition

Amylase inhibitory effects of ethyl acetate, methanol and water extracts of SC leaf have been reported previously (Poongunran *et al*., 2016). These extracts were further analysed for the type of inhibition on α-amylase. Initial velocities were measured in terms of micromoles of maltose formed at different concentrations of extracts (10, 20 and 30 μgmL^{-1}) and different concentrations of substrate (0.015, 0.02, 0.03 and 0.06 % w/v). The control experiment was performed without extracts. A double reciprocal plot was prepared using 1/V *versus* 1/S where V is the reaction velocity and S is the substrate concentration. The type (mode) of inhibition of the 3 extracts on α-amylase activity was determined by analysing the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics (Kaseem *et al*., 2013; Sheliya *et al*., 2016).

Measurement of α-amylase activity

Percentage α-amylase inhibition of the extracts in the presence of different concentrations of substrate were measured according to the method by Geethalakshmi *et al*. (2010) with slight modifications (Poongunran *et al*., 2016). In brief, SC extract (40 µL) (final concentrations 10, 20 and 30 μgmL^{-1}) was mixed with 80 µL of 20 mM phosphate buffered saline (pH 6.9) and 40 µL of porcine pancreatic α-amylase (5 $unitmL^{-1}$). Reaction mixtures were pre-incubated for 15 min at 37 ºC. Potato starch was added (40 µL) to the tubes (final concentrations 0.015, 0.02, 0.03 and 0.06 % w/v). Control was carried out in

the absence of plant extract or the standard inhibitor. Test blanks were conducted in the presence of plant extracts without α-amylase. The reaction mixtures were incubated for 3 min at 37 ºC. Dinitrosalicylic acid colour reagent was added (100 µL) to the tubes and immediately placed at 85 ºC for 15 min. Distilled water (900 µL) was added to each tube and the absorbance was read at 540 nm. Initial velocities were measured in terms of micromoles of maltose formed using a maltose standard curve.

Statistical analysis

Inhibitory assays on fructosamine formation and α-glucosidase were performed 3 times in duplicate. Statistical analysis was performed using ANOVA to compare the means of different extracts. Values of $p < 0.01$ were considered as significantly different.

RESULTS AND DISCUSSION

Different parts of *S. cumini* are used to treat diabetes (Helmstadter, 2008; Ediriweera & Ratnasooriya, 2009). However, investigations carried out on the antiglycation and hypoglycaemic effects of SC leaves are limited. Therefore, extracts of SC leaves were investigated for inhibitory effects on fructosamine formation, protein glycation, glycation-induced protein cross-linking, α-glucosidase, and kinetics on amylase inhibitory effects.

Yield of the extracts

Yield of the extracts obtained by sequential fractionation with hexane, ethyl acetate, methanol and water were 2.5, 4.75, 17.25 and 9.5 % (of the weight of dry leaf powder), respectively (Figure 1). Methanol and water extracts were the major fractions recovered while hexane extract had the lowest weight (Figure 1). Yield of the crude methanol extract obtained directly from the other batch of leaf powder was 30.2 % of the dry weight.

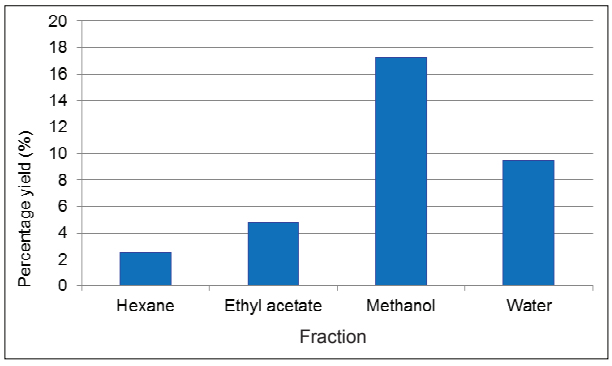

Figure 1: Yield of the SC leaf extracts obtained by differential fractionation. Percentage yield of the four fractions as a percentage of the weight of the dry leaf powder is shown.

Effects of SC extracts on relative fructosamine concentration

The relative concentration of fructosamine was assumed to be proportionate to the difference between the test (T) and the blank (B). T-B of aliquots collected on day 5 of the incubation was compared. T-B of the uninhibited reaction (with no plant extract) was expressed as 100 %. When compared with the uninhibited control, fructosamine formation was significantly inhibited (34.3 to 60.5 %) in the presence of different SC extracts at 2 $mgmL^{-1}$ ($p < 0.0001$). A better inhibition was observed with E, M and W fractions, when the concentration was reduced to 0.25 and 0.05 $mgmL^{-1}$. This could be due to the presence of phytochemicals in E, M and W that can react with nitroblue tetrazolium when high concentrations were used, masking the actual inhibitory effect. C, M and W fractions showed an inhibition > 97 % in the presence of 0.05 $mgmL^{-1}$ (Figure 2). Hexane extract did not show a significant inhibition on fructosamine formation ($p > 0.08$) at 0.05 $mgmL^{-1}$ (Figure 2). AG (1 $mgmL^{-1}$) showed 47.9 % inhibition on fructosamine formation (Figure 2).

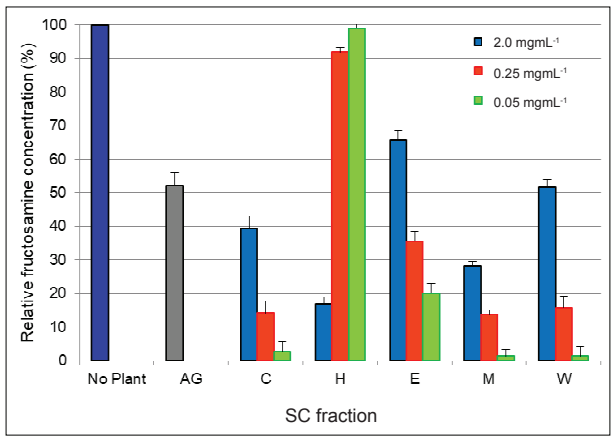

Figure 2: Effects of SC leaf extracts on the formation of fructosamine. Incubations were carried out in the presence of AG: aminoguanidine; C: crude methanol extract; H: hexane extract; E: ethyl acetate extract; M: methanol extract; W: water extract. Concentrations of extracts used were 2, 0.25 and 0.05 $mgmL^{-1}$. SD values are given with each data point.

Effects of SC extracts on glycation

When PAGE was conducted, an increase in BSA migration was observed (downward arrow) with the samples incubated in the presence of fructose (Figure 3). This increase in the movement (compared to that of samples incubated in the absence of fructose) was found to be proportionate to the degree of glycation (Wijetunge & Perera, 2014). The increase in BSA migration was reduced (upward arrow) in the presence of SC extracts indicating glycation inhibition (Figure 3). This inhibition persisted even on day 30 although the standard inhibitor AG did not show much inhibition on day 30 (Figure 3B). Inhibitory effect of the hexane extract was reduced at 0.1 $mgmL^{-1}$, while the inhibitory effects of the other extracts remained the same (Figure 3D). There was interference on the effects seen with E, M and W extracts at higher concentrations (1 and 5 $mgmL^{-1}$) as there was an increase in BSA migration even in the absence of fructose (Figures 3A, 3B, 3C). This could be due to the presence of phytochemicals that can increase the movement of BSA in these three extracts, masking the actual inhibitory effect. Therefore, in order to assess the actual inhibitory effects on fructosamine formation and glycation, it was necessary to bring down the concentration of E, M and W extracts.

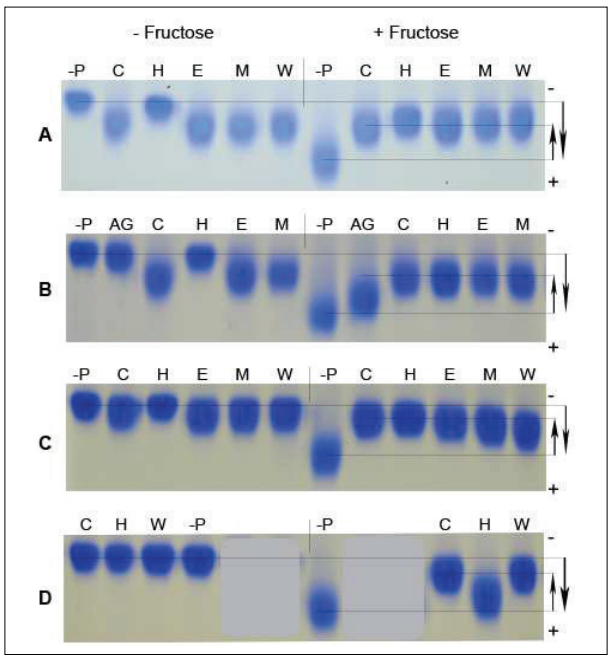

Figure 3: Glycation inhibitory effects of SC leaf extracts
A: at day 12 with 5 $mgmL^{-1}$ extracts (C, H, E, W); B: at day 30 with 5 $mgmL^{-1}$ extracts; C: at day 13 with 1 $mgmL^{-1}$ extracts; D: at day 13 with 0.1 $mgmL^{-1}$ extracts; AG: aminoguanidine; -P: no extract

Effects of SC extracts on glycation induced protein cross-linking

SDS PAGE revealed the formation of high molecular weight products when lysozyme was incubated in the presence of fructose (Figure 4). The amount of high molecular weight products formed was found to be proportionate to the degree of glycation induced protein cross-linking (Perera & Ranasinghe, 2015). The products formed represented the dimer, trimer and tetramer of lysozyme as demonstrated previously using molecular weight markers (Perera & Handuwalage, 2015; Perera & Ranasinghe, 2015). The amount of high molecular weight products formed was reduced in the presence of AG and all SC extracts at 2 $mgmL^{-1}$, indicating inhibition of protein cross-linking (Figure 4). This inhibition remained even on day 21 (Figure 4B). There was no inhibition observed with 0.1 $mgmL^{-1}$ hexane extract while the inhibitory effects remained the same when other extracts were used at 0.1 $mgmL^{-1}$ (Figure 4C). The hexane extract did not show inhibitory effects even at 0.25 $mgmL^{-1}$ (Figure 4A) and 0.5 $mgmL^{-1}$ (Figure 4C). High molecular weight products were not detected in the absence of fructose (results not shown).

Some evidences are reported on the antiglycation effects of SC bark and seed (Perera *et al*., 2013; Tupe *et al*., 2015). Furthermore, the antioxidant potential and the presence of high level of phenolic compounds in SC bark and seed were also reported indicating the likelihood of antiglycation effects in the extracts. Many studies have shown a correlation between the free radical scavenging capacity of plants and glycation inhibitory effects *in vitro* (Dearlove *et al*., 2008; Grzegorczyk-Karolak *et al*., 2016). Phenolic compounds are considered to be the major determinants of antioxidant potential of a test sample (Aberoumand, 2012; Grzegorczyk-Karolak *et al*., 2016). Phenolics have also shown effects against glycoxidation (Sadowska-Bartosz & Bartosz, 2015). The formation of glycoxidation products and cross-linking of collagen was found to be inhibited under antioxidative conditions (Fu *et al*., 1994).

Antiglycation effects of SC bark decoctions were reported by measuring the reduction of relative amounts of fluorescence advanced glycation products in the presence of decoctions (Perera *et al*., 2013). The same study reported the presence of high phenolic content and antioxidant activity in SC bark decoctions. When decoctions of five antidiabetic plants; *Cassia auriculata* (flowers), *Osbeckia octandra* (leaves), *Phyllanthus emblica* (fruits), *Scoparia dulcis* (whole plant) and SC (bark) were tested in another study, the highest content

of phenolic compounds and highest antioxidant and antiglycation effects were detected in the SC decoction (Perera *et al*., 2015). Water extracts of SC seed and *Terminalia bellirica* fruit showed a high level of inhibition on the aggregation of glycated albumin, when the effect of plant extracts were investigated using two amyloid markers (Tupe *et al*., 2015). SC seed methanol extract showed a high antiglycation effect, free radical scavenging activity and high content of polyphenolics and flavonoids (Atale & Rani, 2016). Cell based experiments conducted using H9C2 cardiac cells under high glucose concentration also validated the antiglycation potential of methanol extract of SC seed with no signs of toxicity (Atale & Rani, 2016).

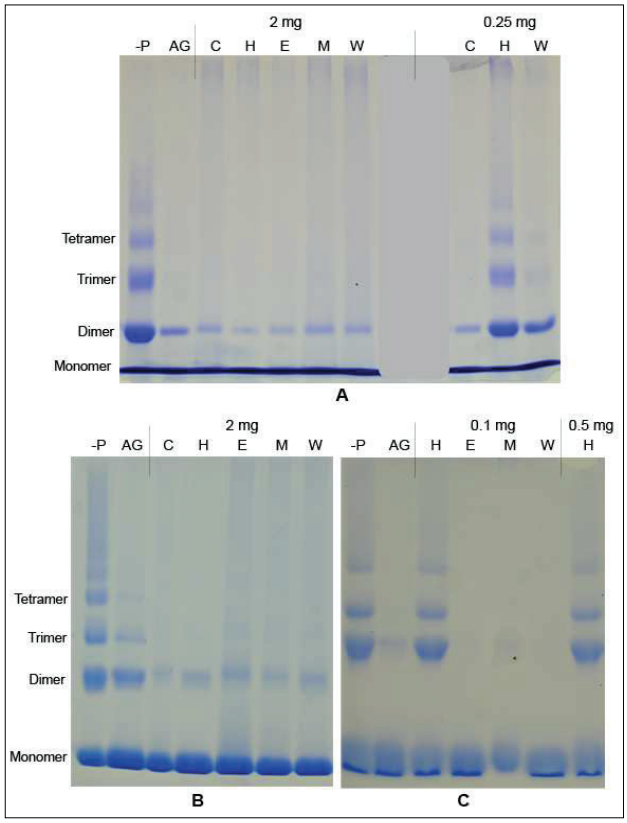

Figure 4: Glycation induced protein cross-linking inhibitory effects of SC leaf extracts
A: at day 5 with 2 and 0.25 mgmL^{-1} extracts (C, H, E, W); B: at day 21 with 2 mgmL^{-1} extracts; C: at day 7 with 0.1 and 0.5 mgmL^{-1} extracts; AG: aminoguanidine; -P: no extract

Antiglycation effects of the SC leaf crude methanol extract were demonstrated recently and the effects were found to be thermostable (Perera & Premadasa, 2016). In the present study all SC leaf extracts investigated including the crude methanol extract showed inhibitory effects on fructosamine formation, glycation and glycation induced protein cross-linking. Hexane extract showed the lowest antiglycation efficacy while the other extracts showed stronger activities as demonstrated using lower concentrations of extracts. Extracts of SC leaves with higher antiglycation effects have shown interference with the measurement of relative fructosamine concentration and assessment of glycation with the PAGE method in the current study. Similar interferences were also observed previously with extracts having stronger antiglycation activities (Perera & Handuwalage, 2015). Some phytochemicals are also reported to interfere with the measurement of fluorescent AGEs (Séro *et al*., 2013).

SC leaves are reported to be rich in polyphenolic compounds and demonstrated antioxidant capacity. A higher polyphenolic content was found when the SC leaf hydroethanolic extract was analysed in comparison to those described for SC seed and fruit (Scanches

et al., 2016). The same leaf extract has also shown a good antioxidant capacity (Scanches *et al*., 2016). In agreement with these findings, another study showed that the water extract of SC leaves has the highest phenolic content when compared with n-hexane, chloroform, ethyl acetate, n-butanol, methanol and water extracts of SC bark, leaf and seeds (Haroon *et al*., 2015). Among these eighteen extracts the third highest content of phenolics was found in the methanol extract of SC leaves (Haroon *et al*., 2015). High antiglycation effects seen in the methanol and water extracts of SC leaves in the present study may be at least partly attributed to the higher phenolic content reported to have in these extracts. A great correlation was demonstrated between the antiglycation activity and total flavonoid content (Grzegorczyk-Karolak *et al*., 2016). SC leaf is reported to be rich in flavonoids such as myricetin, myricitrin, quercetin, kaempferol, ellagic acid, ferulic acid and gallic acid (Ruan *et al*., 2008; Ayyanar & Subash-Babu, 2012). Ellagitannins and gallotannins also were identified from the SC leaf ethanolic extract (Sanches *et al*., 2016).

Effects of SC extracts on α-glucosidase activity

Very high α-glucosidase inhibitory effects were detected in all SC fractions with IC_{50} values < 8 μgmL^{-1} (Table 1). A significantly higher inhibitory potential was observed in methanol and water extracts while the lowest effect was detected in the hexane extract. Glucosidase inhibitory activities between the fractions other than that of methanol and water extracts ($p > 0.8$) were significantly different ($p < 0.001$ to 0.00001) when 10 μgmL^{-1} extracts were used.

Percentage inhibitions of α-glucosidase activity by different concentrations of four SC extracts are shown in Figure 5. The inhibitory effects were rapidly increased with increasing concentrations of methanol and water extracts.

Table 1: α-glucosidase inhibitory effects of SC leaf extracts

SC Extract	% Inhibition (10 μgmL^{-1})	IC_{50} (μgmL^{-1})
Hexane	57.49 ± 1.45	7.89
Ethyl acetate	80.43 ± 2.29	3.11
Methanol	98.75 ± 0.97	0.86
Water	98.87 ± 1.13	0.69
Acarbose		210.54

n = 3 (in duplicates)

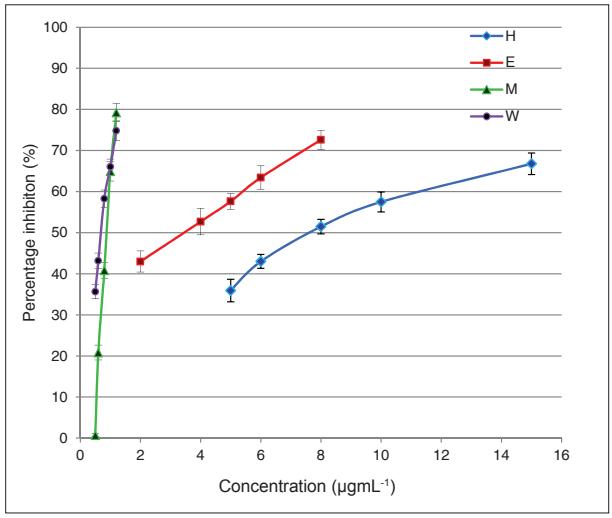

Figure 5: Percentage inhibitions of α-glucosidase activity by different concentrations of SC leaf extracts.
SD values are given with each data point.

α-glucosidase inhibitory effects were reported in SC seed extract (Shinde *et al*., 2008). The α-glucosidase inhibitor isolated from SC seeds was identified as a flavonoid apigenin 7-*O*-glucoside (Alagesan *et al*., 2012b). α-glucosidase inhibitory activity was also demonstrated in the ethanol extract of SC leaves with an IC_{50} of 17.4 μgmL^{-1} (Saraswathy, 2012). In the present study, all extracts showed strong α-glucosidase inhibitory effects (IC_{50} < 8.0 μgmL^{-1}). Furthermore, significantly higher activities were detected in the methanol and water fractions of SC leaves while the lowest activity was reported in the hexane extract.

Kinetics of α-amylase inhibition

In the present study kinetics of amylase inhibition was analysed. Initial velocities were measured in terms of micromoles of maltose formed at different concentrations (10, 20 and 30 μgmL^{-1}) of SC leaf extracts and different concentrations of substrate (0.015, 0.02, 0.03 and 0.06 % w/v). The hexane fraction did not inhibit amylase. α-amylase inhibitory effects of ethyl acetate, methanol and water fractions of SC leaves were previously reported (Poongunran *et al*., 2016). However, kinetics of these inhibitory effects were not studied previously. Results on amylase inhibitory effects of the present study were in agreement with the results of Poongunran *et al*. (2016). Lineweaver-Burk plots were produced with the data obtained in the presence of ethyl acetate (Figure 6A), methanol (Figure 6B) and water (Figure 6C) extracts of SC leaves. The results suggest

mixed type inhibitory effects on α-amylase by the three extracts as K_m and V_{max} differed from those of the control sample [in the absence of inhibitory effects (0)]. These differences were more distinguished in the presence of methanol and water extracts indicating greater inhibition (Figures 6B, 6C).

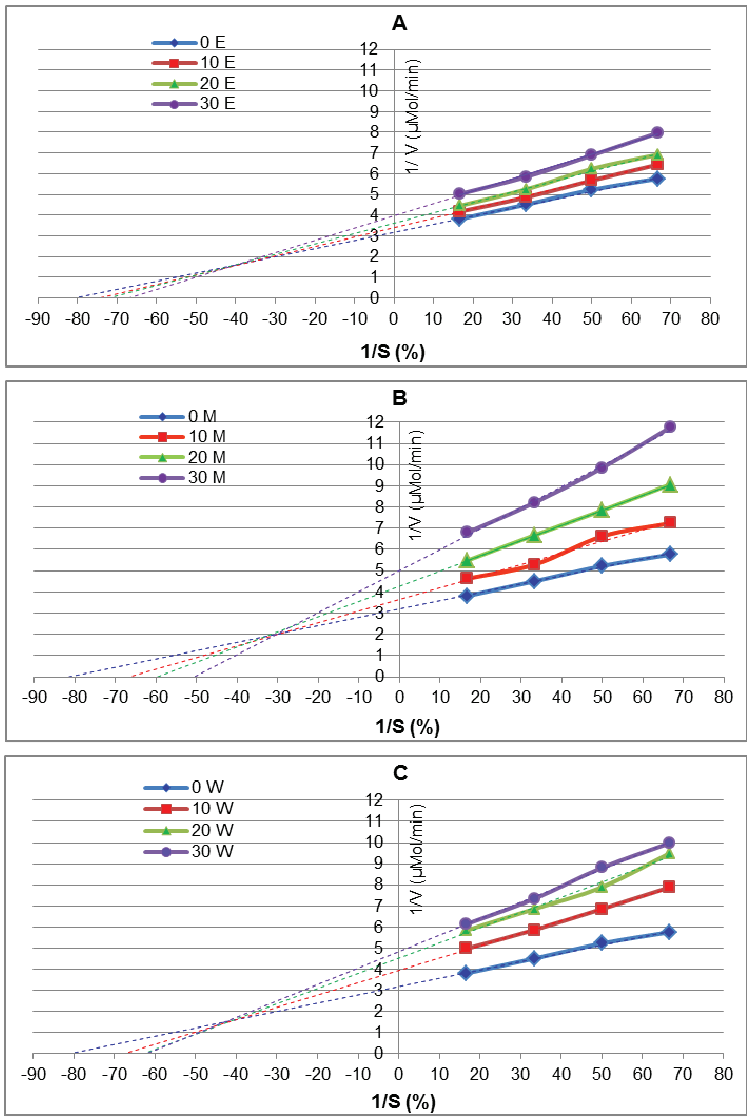

Figure 6: Lineweaver–Burk plot analysis of the inhibition kinetics of α-amylase inhibitory effects by SC leaf extracts
A: with ethyl acetate extract; B: with methanol extract; C: with water extract

α-amylase inhibitory activities were reported recently from the ethyl acetate, methanol and water fractions of SC leaves with IC_{50} values of 704, 61 and 48.3, respectively while revealing no activity in the hexane extract (Poongunran *et al*., 2016). Findings of the kinetics in present study indicate that the inhibitory effects of the three extracts are of mixed type as both V_{max} and K_m values were altered. Hence the respective inhibitors may have the ability to retard the catalysis by binding to the free enzyme as well as to the enzyme-substrate complex. In agreement with the previous findings of Poongunran *et al*. (2016), higher inhibitory effects were observed with methanol and water extracts as the V_{max} values obtained were lower and the K_m values were higher when compared with those of ethyl acetate extracts.

CONCLUSION

The present study demonstrates the antiglycation and hypoglycaemic effects of SC leaves providing scientific evidence to validate its use for antidiabetic effects. Comparatively higher α-glucosidase and α-amylase inhibitory activities were detected in the methanol and water extracts, which were the major fractions recovered with a higher yield (50.74 % and 27.94 % of the total yield, respectively). Hexane extract (smallest fraction) showed the lowest inhibitory effects on the α-glucosidase, fructosamine formation, protein glycation and glycation induced protein cross-linking. Other extracts showed strong antiglycation effects at concentrations as low as 0.1 $mgmL^{-1}$. The hexane extract did not inhibit α-amylase as reported in a previous study. Ethyl acetate, methanol and water extracts exhibited a mixed type of inhibition on α-amylase. Further studies are necessary to reveal the efficacy and safety of SC leaf extracts *in vivo* and to identify the active compounds.

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